Cellular plasticity in liver regeneration - spotlight on cholangiocytes

HEPATOLOGY

Cellular plasticity in liver regeneration - cholangiocytes take centre stage.

Journal:	*Hepatology*
Manuscript ID	HEP-18-1688
Wiley - Manuscript type:	Hepatology Elsewhere
Date Submitted by the Author:	22-Aug-2018
Complete List of Authors:	Tirnitz-Parker, Janina; Curtin University, School of Biomedical Sciences; University of Western Australia, School of Medicine and Pharmacology, Fremantle Hospital Forbes, Stuart; Edinburgh University, MEDICINE Olynyk, John; Fremantle Hospital, Gastroenterology Ramm, Grant; QIMR Berghofer Medical Research Institute, ; The University of Queensland, Faculty of Medicine and Biomedical Sciences
Keywords:	liver regeneration, cellular plasticity, chronic liver disease, transdifferentiation

Cellular plasticity in liver regeneration - cholangiocytes take centre stage.

Janina E. E. Tirnitz-Parker,[1,2,*] Stuart J. Forbes,[3] John K. Olynyk,[4,5] Grant A. Ramm[6,7]

[1]School of Pharmacy and Biomedical Sciences and Curtin Health Innovation Institute, Curtin University, Perth, WA, Australia

[2]School of Medicine and Pharmacy, University of Western Australia, Fremantle, WA, Australia

[3]MRC Centre for Regenerative Medicine, Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK

[4]Department of Gastroenterology, Fiona Stanley Fremantle Hospital Group, Murdoch, WA, Australia

[5]School of Medicine and Health Sciences, Edith Cowan University, Joondalup, WA, Australia

[6]QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

[7]Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia

*corresponding author:
Nina Tirnitz-Parker, N.Tirnitz-Parker@curtin.edu.au, +61-8-9266-9695.

Word count, including references: 1476 words.

The liver's remarkable capacity to self-repair and regenerate following tissue injury has been recognised since the ancient Greek myth of Prometheus. However the diverse potential sources of this regenerative capacity have been an area of hot debate and only recently have studies started to unravel the actual degree of hepatic cell plasticity. The article by **Deng X, Zhang X, Li W *et al.* Chronic liver injury induces conversion of biliary epithelial cells into hepatocytes. *Cell Stem Cell* 2018; 23:114-122** established through lineage tracing experiments using a double-fluorescent reporter system that biliary epithelial cells significantly contributed to hepatocyte regeneration in two murine chronic liver injury models. Furthermore, during the cholangiocyte-to-hepatocyte conversion, bi-phenotypic cells were identified in both mouse models as well as in human cirrhotic livers. Following analysis of liver progenitor cell markers and mature cholangiocytes, the authors concluded that cholangiocytes directly lineage-converted to hepatocytes without a progenitor cell intermediate and suggested these bi-phenotypic cells as potential cellular sources for future therapeutic transplantation strategies.

The landscape of published evidence supporting the regenerative capacity and plasticity of hepatocytes and cholangiocytes has changed rapidly over the last few years and a novel working model is gradually emerging, describing several potential routes to liver regeneration. It involves (a) lineage-restricted regeneration, where mature hepatocytes or biliary epithelial cells proliferate and generate new hepatocytes or cholangiocytes, respectively, or (b) non-lineage-restricted regeneration, mediated by immature, bipotential liver progenitor cells (LPCs), giving rise to either of the two main hepatic epithelial lineages, or - only recently fully recognised - 'transdifferentiation-based regeneration', where mature hepatocytes or cholangiocytes convert to the opposite lineage to replace lost tissue.

Several recent papers have indicated the heterogeneity of hepatocytes in both the physiological maintenance of liver mass and following injury.[1-3] Of note, a periportal source of regenerative hepatocytes was described. These hybrid hepatocytes (HybHP), located at the limiting plate, were positive for both hepatocyte nuclear factor 4 α (HNF4α) and the ductal transcription factor Sox9 but were negative for the cholangiocyte and liver progenitor cell marker cytokeratin 19 (CK19). HybHPs regenerated hepatocytes following chronic and carcinogenic injury.[1] Hepatocyte-to-

cholangiocyte transdifferentiation has been shown by Schaub *et al*. in a mouse model of Alagille syndrome that results in cholestatic injury at birth and leads to postnatal *de novo* cholangiocyte formation. This cellular plasticity was mediated via transforming growth factor β signalling.[4] In this model, there is likely a strong selective pressure on hepatocytes due to the lack of pre-existing peripheral bile ducts. Similarly, selective pressures facilitated the significant transdifferentiation of biliary epithelial cells to hepatocytes following substantial hepatocyte depletion using zebrafish as the model organism.[5] For some time it had been controversial whether cholangiocyte-to-hepatocyte conversion was an effective mechanism of hepatocyte regeneration in zebrafish alone or whether it also occurred in mouse and humans. Experiments in mice, combining significant liver injury with the inhibition of hepatocyte proliferation by either knockdown of the transmembrane heterodimeric protein β1-integrin or overexpression of the cyclin-dependent kinase inhibitor p21 in hepatocytes, led to induction of cholangiocyte-derived ductular reactions and the formation of functional, biliary epithelial cell-derived hepatocytes.[6] Together these data have demonstrated functionally relevant cellular plasticity in the epithelial compartment of the injured liver, adding cholangiocytes to the list of potential cell sources for hepatocyte regeneration and *visa versa*.

In a recent issue of *Cell Stem Cell*, Deng *et al*. corroborated and extended these findings by confirming cholangiocyte-to-hepatocyte transdifferentiation in murine lineage tracing models in the absence of genetic interventions.[7] The authors used thioacetamide (TAA) administration as a model of progressive fibrosis and cirrhosis and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) as a model of cholangitis and biliary fibrosis. The study took advantage of double-fluorescent Cre reporter mice, which displayed global expression of membrane-targeted tandem dimer Tomato, unless excision had taken place through the expression of Cre recombinase. The authors achieved this in hepatocytes using an adeno-associated virus expressing Cre recombinase under the control of the hepatocyte-specific thyroxine-binding globulin promoter. Hence, cells ubiquitously fluoresced red except for adult hepatocytes, which exhibited green fluorescent protein (GFP) expression. In both injury models, following extended injury, patches of HNF4α^{+} hepatocytes developed that were red (i.e. were not Cre-deleted), suggesting a non-hepatocyte origin. However, in this system the authors cannot formally exclude the possibility that small numbers of

hepatocytes that escaped the Cre-deletion expanded throughout the parenchyma. However, this scenario is not considered likely as any putative Cre-escaped hepatocytes would not have any known selective advantage over the Cre-deleted hepatocytes. Importantly, following TAA-injury, putative transdifferentiated hepatocytes exhibited markers of spatial zonation, including expression of carbamoyl phosphate synthetase I in periportal and glutamine synthetase in pericentral areas, and were therefore demonstrated to have functionally integrated into the liver parenchyma. In addition, none of the liver cancers that formed after 52-week TAA treatment were derived from transdifferentiated hepatocytes. To formally prove the non-parenchymal origin of these new hepatocytes, Deng *et al*. used positive lineage tracing. Co-staining of HNF4α and CK19 revealed double-positive cells in periportal liver areas. Lineage tracing for $CK19^{+}$ biliary epithelial cells was performed, which revealed that these cells migrated from ductal to parenchymal areas, adopted hepatocyte shape and consequently expressed HNF4α, cytochrome P450 3A4 and multidrug resistance protein 4. Furthermore, these cholangiocyte-derived cells lacked expression of CK19 and Sox9. It was estimated that approximately 9-10% of hepatocytes were derived through hepatocyte transdifferentiation of biliary epithelial cells in the TAA- and DDC-induced liver injuries. Bi-phenotypic cells that co-expressed HNF4α and CK19 and exhibited columnar and stratified epithelial morphology were further analysed. The majority of bi-phenotypic cells lacked primary cilia as well as expression of the polarity marker protein kinase C zeta, suggesting that cells had lost their typical apical-basal polarity during the conversion process. A few mature cholangiocytes displayed co-expression of HNF4α and primary cilia, which prompted the authors to propose that cholangiocyte-to-hepatocyte transdifferentiation was the result of a previously unrecognised direct lineage conversion. The fact that bi-phenotypic cells did not express the liver progenitor cell markers Lgr5 and alpha-fetoprotein led *Deng et al*. to conclude that this conversion had taken place without a liver progenitor cell intermediate. It should be noted that (a) the putative liver progenitor cell pool consists of a very heterogeneous cell population, which necessitates the simultaneous use of multiple markers to identify all subpopulations and (b) alpha-fetoprotein is only suitable as a liver progenitor cell marker in rats and not in mice.[8] Therefore, a more detailed analysis of these bi-phenotypic cells would need to be undertaken before the

conclusion of a direct cholangiocyte-to-hepatocyte lineage conversion without a progenitor cell intermediate can be formally proven.

While underlying mechanisms may vary between species and are certainly context-specific, these data emphasise that the liver has an abundance of effective regenerative sources, inducing the most appropriate cell in a given injury scenario. Collectively, these recent papers[5, 8, 9] have highlighted the plasticity of the liver's epithelial cell population, and clearly shown that this response is dependent upon the type of injury and regenerative failure of the 'native epithelial cell'. This has also helped to uphold the liver's reputation as a regeneration super star among solid organs.

References

1. Font-Burgada J, Shalapour S, Ramaswamy S, Hsueh B, Rossell D, Umemura A, Taniguchi K, et al. Hybrid Periportal Hepatocytes Regenerate the Injured Liver without Giving Rise to Cancer. Cell 2015;162:766-779.
2. Lin S, Nascimento EM, Gajera CR, Chen L, Neuhofer P, Garbuzov A, Wang S, et al. Distributed hepatocytes expressing telomerase repopulate the liver in homeostasis and injury. Nature 2018;556:244-248.
3. Planas-Paz L, Orsini V, Boulter L, Calabrese D, Pikiolek M, Nigsch F, Xie Y, et al. The RSPO-LGR4/5-ZNRF3/RNF43 module controls liver zonation and size. Nat Cell Biol 2016;18:467-479.
4. Schaub JR, Huppert KA, Kurial SNT, Hsu BY, Cast AE, Donnelly B, Karns RA, et al. De novo formation of the biliary system by TGFbeta-mediated hepatocyte transdifferentiation. Nature 2018;557:247-251.
5. Choi TY, Ninov N, Stainier DY, Shin D. Extensive conversion of hepatic biliary epithelial cells to hepatocytes after near total loss of hepatocytes in zebrafish. Gastroenterology 2014;146:776-788.
6. Raven A, Lu WY, Man TY, Ferreira-Gonzalez S, O'Duibhir E, Dwyer BJ, Thomson JP, et al. Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. Nature 2017;547:350-354.
7. Deng X, Zhang X, Li W, Feng RX, Li L, Yi GR, Zhang XN, et al. Chronic Liver Injury Induces Conversion of Biliary Epithelial Cells into Hepatocytes. Cell Stem Cell 2018;23:114-122 e113.
8. Jelnes P, Santoni-Rugiu E, Rasmussen M, Friis SL, Nielsen JH, Tygstrup N, Bisgaard HC. Remarkable heterogeneity displayed by oval cells in rat and mouse models of stem cell-mediated liver regeneration. Hepatology 2007;45:1462-1470.

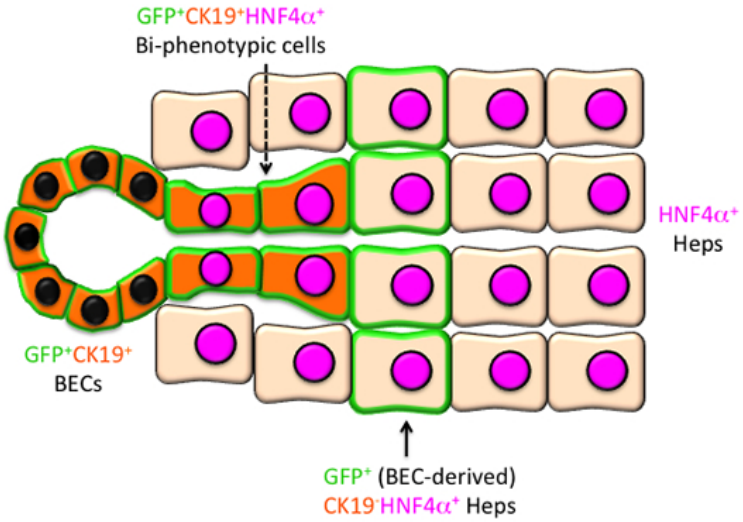

Figure 1: Biliary epithelial cell-to-hepatocyte conversion.

60x44mm (300 x 300 DPI)

Figure 1: Biliary epithelial cell-to-hepatocyte conversion. Deng *et al*. used tamoxifen treatment in $CK19^{CreERT}$:mTmG mice to label cytokeratin 19 (CK19)-expressing biliary epithelial cells (BECs) with green fluorescent protein (GFP). Chronic liver injury through treatment with thioacetamide or 3,5-diethoxycarbonyl-1,4-dihydrocollidine led to the generation of bi-phenotypic cells that co-expressed CK19 and hepatocyte nuclear factor α (HNF4α). In addition, single-cell lineage tracing was performed in thioacetamide-treated $CK19^{CreERT}$:mTmG mice through one round of low-dose tamoxifen administration. Single GFP^{+} BECs gave rise to small hepatic nodules of $CK19^{-}HNF4\alpha^{+}$ cells, providing evidence for a BEC-to-hepatocyte conversion.